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Scanning electron microscopy analysis of sol-gel derived biocompatible glass

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Abstract. Bioactive silica gels / polymer systems have been produced using a sol-gel route and their bio-compatibility has been investigated by immersing them in simulated body fluid (SBF). The porous monoliths have been characterised by SEM and EDX analysis where images obtained show pores on the surface of 10 - 200 μm. The silica gels are not homogeneous and distinct regions of silicon and calcium are observed. The growth of an apatite layer on the surface of the gels was evident after steeping in SBF.

1. Introduction

Bioactive glasses have been the subject of various studies in recent years due to their ability to bond to living tissues by inducing the formation of a hydroxyapatite layer on the surface in the presence of biological fluids [1-3]. The porosity of the bioactive gels can be controlled in the manufacturing process. Most methods for preparing bioactive glasses involve creating the matrix via a sintering phase at high temperatures [4]. We have been investigating a 'low' temperature method using a sol-gel process to avoid problems associated with temperature degradation when incorporating biological materials during matrix production [5].

The formation of a dense apatite layer *in vitro* is believed to mimic the process *in vivo* and be an indication of the bioactivity of the artificial material. This is believed to be an essential requirement for the artificial material to bond to living bone. The apatite growth on the surface of the material is due to calcium and phosphate ions being absorbed from the surrounding fluid to form a dense layer on the surface of the material [6,7].

The aim of this work is to monitor the pore sizes obtained for the bioactive glass manufactured using the 'low' temperature (sol-gel) method and determine the effect of the different constituent components, including the inclusion of the biocompatible polymers, polyethylene glycol (PEG) (several molecular weights), polymethyl methacrylate (PMMA) and polyethylene (PE) in the manufacturing process. Analysis of the surface porosity will be undertaken using SEM studies and elemental analysis using energy dispersive X-ray analysis (EDX). In order to determine if an apatite

layer will grow on the surface of the silica gels in this study the sol-gels have been steeped in simulated body fluid (SBF).

2. Experimental

All chemicals were purchased from commercial chemical suppliers and used as received. Low temperature bioactive silica gels were prepared following method 2 outlined by Hall *et al* [4]. Briefly, calcium nitrate ($Ca(NO_3)_2.4H_2O$, 4.72 g) which was acidified in nitric acid (HNO_3, 1M, 12ml) was added to tetraethyloxysilane (TEOS, 16.67 g) and stirred for 30 min. Calcium carbonate ($CaCO_3$, 4 g) and baking powder (1 g) were then added prior to the mixture being poured into a mould and heated at 180 ^oC for 30 - 40 min. This was the standard method of sample preparation. Some samples were then treated with 1 M HCl, (indicated by (HCl)) in order to attempt the removal of the added calcium carbonate and promote sample porosity. One sample was prepared with 2 g of baking powder.

Hybrid matrices were prepared by either adding 2 g of PEG (MWt. 400 or 1000), PMMA or low density PE to the mixture at the early gelation stage or prepared standard samples were dipped into a monomer solution of PE or PEG1000 in toluene, dried, put in a vacuum for 1.5 hours then, still under vacuum, heated at 180 ^oC for 40 minutes. These samples are identified by vacuum, in brackets.

All samples were prepared as porous and opaque monoliths (~ 7 × 7 × 15 mm). Hybrid matrices, prepared with the addition of polymer will be referred to as Si/Ca/POL. For example, Si/Ca/PE represents the sample prepared with the addition of PE.

Simulated body fluid (SBF) was prepared following the method outlined by Cho *et al* [8] by dissolving the following chemical reagents added in order to 750 ml ultra-pure water at 37^oC: NaCl (7.996 g), $NaHCO_3$ (0.350 g), KCl (0.224 g), $K_2HPO_4.3H_2O$ (0.228 g), $MgCl_2.6H_2O$ (0.305 g), dilute HCl (40 ml), $CaCl_2$ (0.278 g), $NaSO_4$ (0.071 g), $(CH_2OH)_3CNH_2$ (6.057 g). HCl was then added to adjust the pH to 7.25. The solution was then allowed to cool to room temperature before diluting to 1000 ml. Bioactive silica gels were then added to 20 ml of SBF and soaked at 37 ^oC in a controlled environment for the specified length of time after which the samples were rinsed with ultra-pure water and dried in a desiccator for 48 h before being coated for SEM analysis.

Samples for SEM analysis were sputter coated with Au/Pd using a Polaron SC7640 sputter coater. Scanning Electron Microscopy (SEM) images (SE and BSE) of the sample surface were obtained using an EVO-50XVP (Carl Zeiss SMT Ltd). Chemical composition was determined using the Genesis 4000 EDX detector (EDAX Inc.).

3. Results

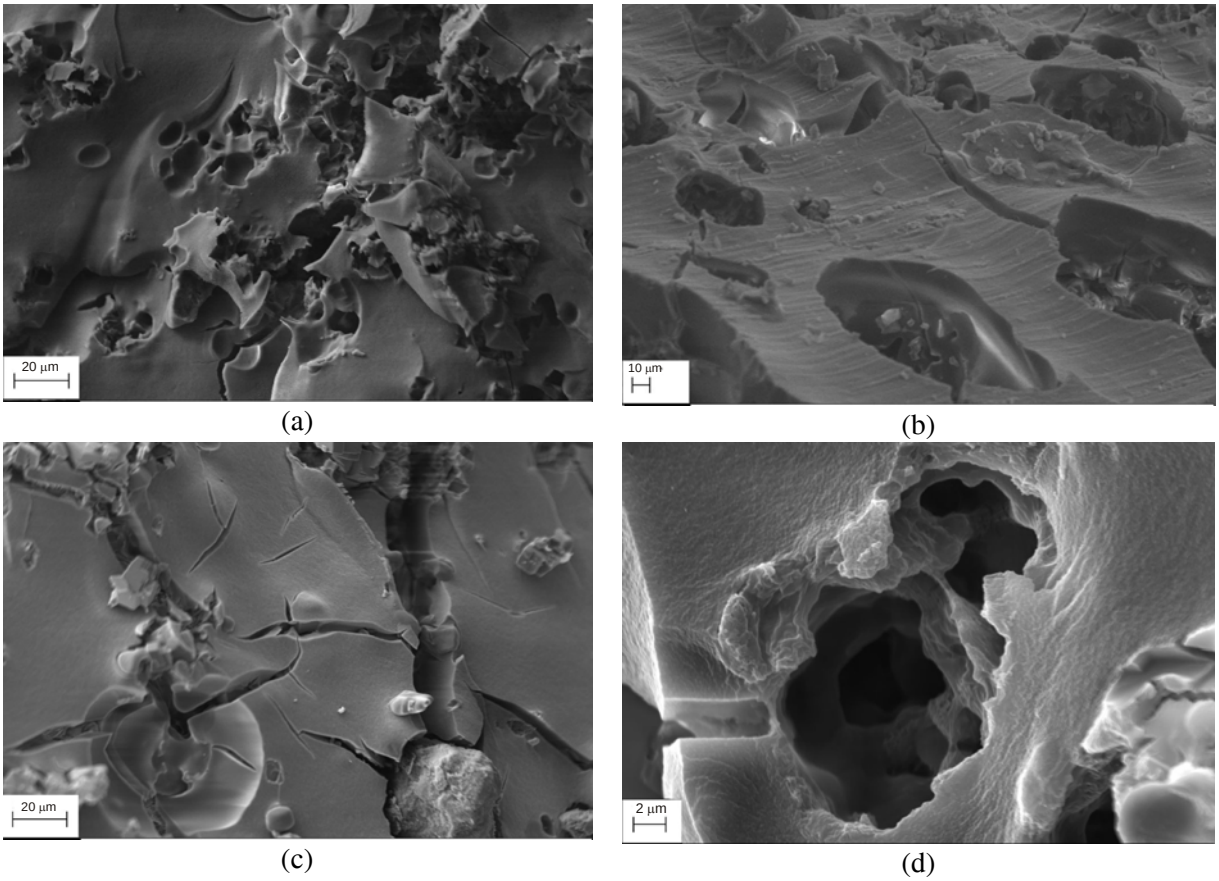
3.1. SEM and EDX analysis of bioactive silica gels.

Figure 1 (a) and (b) compares the secondary electron image of the Si/Ca (HCl) monolith prepared using 1 g and 2 g of baking powder. The sample with 1 g of baking powder has a range of pores of diameter of the order of 10 μm visible on the surface. The addition of an increased amount of baking powder increases the pore dimensions 10 fold. The heat treatment of the monoliths after pouring into the mould appeared to increase their strength and produce samples which were not as brittle.

The addition of polymer to the sample did affect the strength of the material in that the sol-gel was not as prone to crumbling. This is particularly true for the samples which were dip coated and heated under vacuum. Figure 1 (c) and (d) shows the SE image of Si/Ca/PEG1000.

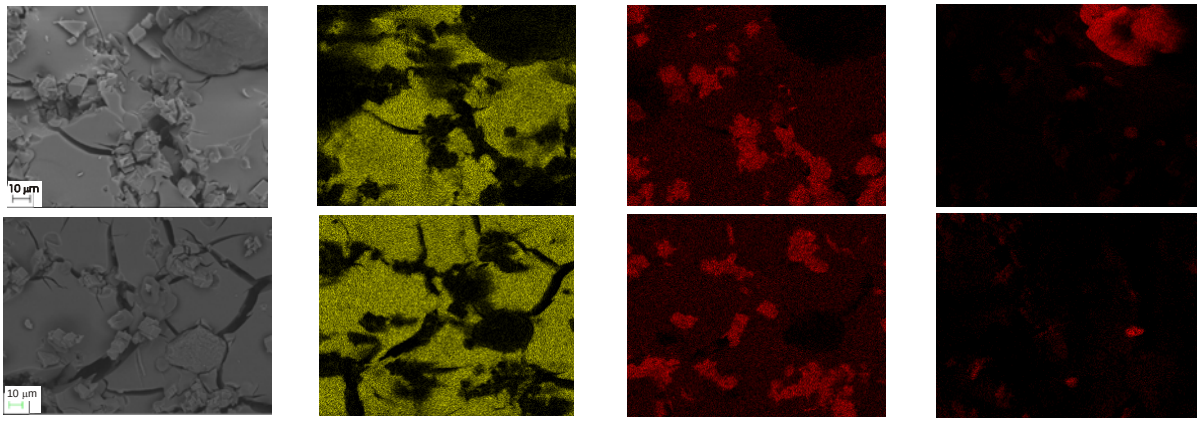
The images in Figure 1 clearly show areas that are smooth in nature and regions where a cubic structure is noted. EDX mapping was carried out to determine the elemental composition of these distinctive areas. Elemental maps showing the areas of Si, Ca and C X-Ray emission are presented for Si/Ca/PE and Si/Ca/PEG400 in Figure 2. The maps clearly show that Si and Ca are not homogeneously mixed throughout the polymer but occupy distinct regions. C found in the Ca regions is consistent with these areas representing $CaCO_3$. Where carbon is found in the absence of Ca, this area is thought to represent the polymer regions. For example, in Figure 2(d), Si/Ca/PE there is an area of carbon in the top right hand corner indicating that this region is likely to be PE. Similar maps

have been obtained for the other monoliths in this study, although in the other cases large areas of carbon were not as evident. Distinct areas of Ca and Si were also confirmed from XRD studies on the samples [9].



(a) (b) (c) (d)

Figure 1: Secondary electron images of (a) Si/Ca (HCl), standard sample, EHT 15 kV (b) Si/Ca, 2 g baking powder, EHT 15 kV, Variable Pressure (c)/Si/Ca/PEG1000, EHT 10kV (d) Si/Ca/PEG1000 (in vacuum), EHT 15 kV



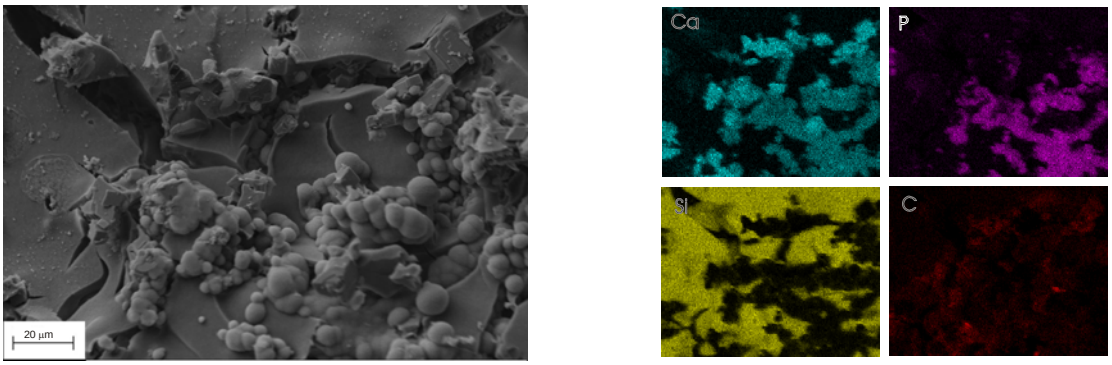
(a) (b) (c) (d)

Figure 2: EDX maps of (top) Si/Ca/PE and (bottom) Si/Ca/PEG400. (a) BSE image EHT 15 kV, (b) Si, (c) Ca, (d) C.

3.2. Samples steeped in SBF

After steeping in SBF for 1 day there was evidence of apatite growth on the surface of all the bioactive gels. This can be seen as spherical growth of radius 5 - 10 μm on the surface of the silica-gel. Figure 3(a) shows the SE image for the Si/Ca/PE (Vacuum) monolith which has been soaked for 6 days. Figure 3(b) shows the elemental maps for Ca, P, Si and C for this monolith. From the X-ray maps it is clear that the areas where Ca and P coincide correspond to the spherical regions in Figure 3(a). Regions where Ca is present, with no P, are calcite regions where no apatite has grown.

EDX spot analysis on one of the spheres confirms that the feature is comprised mainly of Ca and P, the main constitutes of apatite growth. The weight % of Ca/P was found to be 1.3 and is consistent with the composition for apatite growth in other bioactive silica gel matrices [7].



(a) (b)

Figure 3: Si/Ca/PE (Vacuum) soaked in SBF for 6 days (a) SE image, EHT 10 kV (b) EDX element maps, EHT 15 kV.

4. Conclusion

The SEM/EDX results presented show that porous bioactive silica gels, which have been strengthened by the addition of biocompatible polymers, have been produced using a low temperature sol-gel method. Spherical calcium phosphate growths can be seen on the surface of all the monoliths which have been steeped in SBF at 37 ^{o}C.

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